THE EMBRYO SAC AND EMBRYO OF STRIGA LUTEA

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(WITH PLATES VIII AND IX)

Attention lately has been drawn to *Striga lutea*, a semi-parasitic plant belonging to the Rhinanthoideae-Gerardieae (8) group of the Scrophulariaceae, owing to the ravages caused by it in the maize crops in parts of South Africa.

The material for this investigation was obtained by Dr. H. H. W. PEARSON in Pretoria during the autumn of the years 1912 and 1913.

The ovaries were fixed in a chromacetic solution and the chief stain used was Haidenhain's iron-hematoxylin. A combination of diamant fuchsin and light green also gave good results, and Flemming's triple stain gave excellent differentiation in the embryonic stages.

Ovule and embryo sac

There is nothing striking in the ovary. It is of the ordinary bilocular scrophulariaceous type, and bears a large number of minute anatropous ovules on the rather swollen placentae. Many of the ovules possess long funicles, and in some cases the funicle branches and bears two ovules. The fact that the length of funicle varies enables the plant to produce a greater number of ovules per unit area of placenta than it would be able to do were the funicles all of one length.

The archesporium can be distinguished at an early stage before the integument arises. It consists of a single hypodermal cell which, without undergoing division, becomes directly the megaspore mother cell. This is shown in fig. 1, which also shows the origin of the integument. The young ovule grows with great rapidity, and before the first division of the nucleus of the megaspore mother cell, the integument is well marked and the whole ovule is rapidly assuming its mature anatropous form.

The nucellus consists of one layer of cells. As development proceeds the cells become flattened and finally disorganized, so that

when the embryo sac has reached maturity, only traces of it are to be found lying between the integument and the embryo sac.

The bulk of the ovule is composed of the thick integument, as is often the case in the Scrophulariaceae. Fig. 2 represents a transverse section through the ovule just above the integument and shows the nucleus of the mother cell in synapsis, prior to its first division. In the ovule drawn the nucleolus is just visible, but in many similar ovules it has disappeared. It was observed that, when synapsis occurs, about 10 per cent of the ovules in an ovary are in this stage simultaneously. Other stages in the heterotypic division were not seen, the next stage being that of the homotypic division (fig. 3). Fig. 4 shows the three upper megaspores degenerating, while the fourth has become the embryo sac.

The development of the embryo sac is perfectly normal. The nucleus divides and the resulting nuclei pass one to each pole of the embryo sac, the center being occupied by a large vacuole (fig. 5). These nuclei divide twice (figs. 6–8), thus giving two groups of four nuclei, one at each end of the sac. One nucleus from each group then moves toward the center (fig. 9); these two nuclei meet and fuse in the upper part of the sac, not far from the egg cell (fig. 10). BALICKA-IWANOSKA (1) found in certain genera of the Scrophulariaceae that the polar nuclei fuse about the middle of the embryo sac and migrate toward the egg at the time of fertilization. SCHMID (13) pointed out that this position of the polar nuclei at the time of fusion is not that always found in this family. He found that the position at the time of fusion may vary in a single species. For instance, in *Pedicularis palustris* the polar nuclei may fuse in the upper, lower, or middle part of the sac. In the cases in which fusion occurs in the middle or at the base of the sac, they migrate toward the egg at the time of fertilization.

The synergids have assumed a caplike appearance by the time the embryo sac is ready for fertilization. In the embryo sac shown in fig. 11 this cap is not yet developed, but the synergids are early distinguished from the egg by their much smaller size. In this figure the antipodals show signs of disintegration, and at the stage represented in fig. 12 a small deeply staining mass is all that is left of them.

In general behavior this embryo sac differs in no way from the rest of the Scrophulariaceae, in which it is a general rule that the synergids are well marked off from the other nuclei of the embryo sac, and the antipodals are inconspicuous, disappearing about the time of maturity.

Case of an ovule with two embryo sacs

In an ovary in which most of the ovules were in the 4-nucleate or 8-nucleate stages, one ovule was seen showing two sacs lying side by side. Fig. 18 represents a section through this ovule not passing through the embryo sacs. In this section the ovule has every appearance of having been derived from two fused ovules. Figs. 16 and 17 support this view, as the cells separating the two embryo sacs clearly belong to the integument. Unfortunately the sections through this ovule are oblique. Figs. 13–17 are drawn from this ovule as it was represented in five consecutive sections. The left-hand embryo sac has four nuclei arranged in two groups of two each. The right-hand embryo sac contains eight nuclei apparently not definitely arranged in groups. Six of the nuclei lie fairly close together at one end of the sac and two at the other end. Possibly this lack of arrangement is to be correlated with the abnormal conditions under which the sac has developed. This ovule was the only one showing any abnormal tendencies.

Cases in which two ovules have grown together to form one with two embryo sacs have not been reported as frequently as those in which the two embryo sacs arise from a single archesporium. They have been recorded, however, in *Pirus Malus*, *Loranthus europaeus*, and *Viscum album* (5).

Fertilization

Ovules in which fertilization is taking place are fairly abundant. In the section represented in fig. 12 the pollen tube is seen having penetrated the embryo sac and apparently pushed its way through one of the synergids, and has discharged its contents into the sac. One male nucleus is seen fusing with the egg, while the other is fusing with the nucleus produced by the fusion of the polar nuclei. The male nuclei may be easily distinguished from the nuclei of the

embryo sac by their much smaller size. This case is yet another to be added to the ever-growing list of plants in which double fertilization is known to occur.

Endosperm formation

The endosperm is initiated by cell formation. *Striga lutea* thus conforms with the type largely represented in the Sympetalae and almost universal in parasites and saprophytes (5).

The first division of the primary endosperm nucleus is immediately followed by the formation of a transverse wall, dividing the embryo sac into two chambers (fig. 19). The nucleus of the chalazal chamber divides once, but no wall is formed, and endosperm is never produced in this chamber. Its function is clearly haustorial, for soon after the division of the nucleus the chalazal end of the sac grows down into the integument, and finally curving upward reaches the outermost layer of the integument (fig. 22). Fig. 21 shows a somewhat earlier stage in which the remains of the antipodals are seen at the end of the haustorium.

The nucleus of the micropylar chamber divides three or four times, each division being accompanied by a wall transverse to the long axis of the sac (fig. 20). After this, walls appear in various planes and the original transverse walls are soon obscured. No definite micropylar haustorium is formed, though the cells of the endosperm grow a short distance up the micropyle, surrounding the suspensor, and are probably to be considered as having a haustorial function (fig. 23).

The endosperm cells around the suspensor (fig. 23) and those formed at the base of the original micropylar chamber (fig. 22) have dense protoplasm and stain far more deeply than the rest of the endosperm cells. This may be due to the fact that they are active in passing the food, which is gradually being absorbed from the integument by the haustoria, to the developing embryo. In these ovules there is no distinct tapetal layer round the embryo sac.

In the endosperm formation and in the development of haustoria there is a remarkably close resemblance between *Striga lutea* and *Linaria alpina*. SCHMID (13) in his account of *Linaria alpina* might well be describing *Striga lutea*, the chief difference being that

in *Striga* the chalazal haustorium seems to be slightly longer and
to have a definite upward curve.

Embryo

After fertilization the egg cell does not divide immediately.
Figs. 19 and 20 show the fertilized egg in the resting stage while
endosperm is being formed rapidly.

The first division of the egg is transverse, the lower cell giving
rise to the embryo, the upper to the suspensor, which develops
rapidly and is divided into three or four cells by transverse walls
(fig. 23). The cell of the suspensor nearest the micropyle increases
in length far more rapidly than the others and crushes all the endo-
sperm cells at its apex, thus coming to lie in contact with the
integument. In appearance the proembryo is rather like that of
Physostegia (**14**), and also, though shorter, bears a resemblance to
that of *Myoporum serratum* (**3**). The cross-walls which are present
in the suspensor of *Striga* and *Physostegia* are absent in *Myoporum*.
A difference which becomes marked later in the development of the
proembryo is the appearance of haustoria in *Striga*. These are
chiefly confined to the basal cell, though in one case the cell below
has produced a small lateral haustorium (fig. 26). LLOYD (**10**) in
his account of the Rubiaceae shows that in many members of that
family haustoria are developed from the suspensor, but as far as
the writer has been able to ascertain, this has not been recorded for
the Scrophulariaceae.

The embryonic haustoria are tuberous in form and do not show
the slightest resemblance to those of the endosperm.

The first wall of the embryo proper is formed in a longitudinal
plane (fig. 24) and is followed immediately by another longitudinal
wall at right angles to the first, dividing the embryo into four cells
(fig. 25). A transverse wall is then formed, dividing the embryo
into octants. The next walls are periclinal (fig. 26).

Walls then follow in quick succession, giving rise to a spherical
embryo (fig. 27) on the end of a long suspensor. The mature
embryo is of the ordinary dicotyledonous type (fig. 28) and is sur-
rounded by one row of endosperm cells.

Changes in endosperm and testa

At a very early stage in the formation of the endosperm, the outer wall of the outermost layer of endosperm cells becomes cutinized, and it is clearly impossible that food can pass through to the embryo, which must therefore derive its nourishment through the haustoria.

As the embryo grows, absorbing the food stored in the endosperm and integument, the walls of the outer layer of the endosperm become thickened, but are still composed of cellulose. It is these thickened cells which compose the endosperm in the mature seed. Proteid reserves alone are found, neither starch nor oil being present.

There is no well defined tapetal layer around the endosperm as is the case in most Scrophulariaceae already investigated.

All the food stored in the integument is absorbed by the haustoria and only the outermost layer of the integument persists in the mature seed. This layer undergoes a good deal of change. Its radial walls increase in size, assume a wavy outline, and become lignified. It is to this last fact that the brittleness of the testa is due.

It is a well known fact among farmers that the seed of *Striga lutea* may retain its capacity for germination for several years (11), owing no doubt to the protection afforded by the lignified testa and cutinized outer wall of the endosperm, as well as to the reserves of proteid material in the endosperm. When to this is added the fact that each ovary produces hundreds of seeds, practically every one of which is fertile, it is not difficult to realize why it is that this plant is so difficult to eradicate once it has obtained a hold on a crop.

Discussion

Since the middle of the last century the ovules of the Scrophulariaceae have from time to time been the object of investigation. Among the earlier workers on this subject are to be found DEECKE (6), TULASNE (15), HOFMEISTER (9), CHATIN (4), and others.

It is rather interesting to note that *Pedicularis sylvatica* was in 1855 the subject of a violent controversy between DEECKE and SCHACHT on one hand, and HOFMEISTER, VON MOHL, and TULASNE,

on the other. In 1835 SCHLEIDEN had expounded the theory that
the pollen tube entered the embryo sac and there gave rise to the
embryo, basing his conclusions on observations made on ovules of
plants belonging to various families (5). In 1855 DEECKE (6) in
Pedicularis sylvatica claimed to have seen the pollen tube entering
the sac and developing there into the embryo, and came to the
conclusion that in this plant he had proved beyond all doubt that
SCHLEIDEN'S view as to the origin of the embryo was correct.
SCHACHT (12) confirmed DEECKE'S statement, but HOFMEISTER (9)
proved that what DEECKE had seen and drawn was the proembryo.
He believed those figures in which DEECKE depicted the "pollen
tube" wandering outside the micropyle to be due to roughness in
dissection.

It is extremely interesting to find that the "pollen tube" of
DEECKE bears a striking resemblance to the proembryo of *Striga
lutea*. That it is a proembryo is obvious, since DEECKE shows it
imbedded in the endosperm, and he also figures very clearly struc-
tures which in the light of SCHMID'S work on *Pedicularis* we may
interpret as two lobes of a micropylar haustorium and a chalazal
one. *Striga lutea* possesses a long suspensor which is clearly com-
parable with DEECKE'S pollen tube. In the majority of his figures
the end of the "pollen tube" remote from the embryo forms a
swelling much resembling that shown in the young proembryo of
Striga (fig. 23). In DEECKE'S fig. 16 a case is shown of two "pollen
tubes" entering one ovule. One of these tubes is traced down to
the embryo, the other advances only a short way down the micro-
pyle. The explanation of this phenomenon might be that in
Pedicularis sylvatica, as in *Striga*, the basal cell of the suspensor
produces haustoria and the second "pollen tube" is simply a
haustorium. Of recent years the chief contributions to our
knowledge of the embryo sac of the Scrophulariaceae have been
made by BALICKA-IWANOWSKA (1) in 1899 and SCHMID (13) in
1906.

BALICKA-IWANOWSKA'S work is of a more general character than
SCHMID'S, dealing with several sympetalous families, while SCHMID
confines himself to a number of species selected from genera repre-
senting the three main groups of the Scrophulariaceae: Pseudo-

solaneae, Antirrhineae, and Rhinanthoideae. It is on this last group that most work has been done, though its subdivision Rhinanthoideae-Gerardieae, to which *Striga* belongs, has up to the present not been investigated. For this reason it is of great interest to discover how far *Striga* may be compared with the plants belonging to the Rhinanthoideae-Digitaleae and Rhinanthoideae-Rhinantheae. In all the plants which have been studied the features of the greatest interest are found in the post-fertilization phases of development and in this respect *Striga lutea* is no exception.

"Double fertilization" has been demonstrated in *Linaria vulgaris, Digitalis purpurea, Pedicularis foliosa,* and *Melampyrum silvaticum,* to which may now be added *Striga lutea.*

The development of haustoria and the development of endosperm go hand in hand and therefore may be treated together. SCHMID (**13**) puts the genera he has studied into four groups according to their method of endosperm formation.

In the first he puts *Verbascum, Scrophularia,* and *Digitalis.* In these genera four superposed primary endosperm cells are formed, of which only the two inner ones form the true endosperm, the uppermost and lowermost cells dividing into four and assuming a haustorial function, though in a much less marked degree here than is found in the other groups.

Linaria and *Antirrhinum* constitute a second group. In them a transverse division of the embryo sac occurs. The upper cell gives rise to the endosperm, of which a few cells at the micropylar end function as a haustorium; the lower cell grows out into a tube-like haustorium, in which no cell walls are formed though the original nucleus divides once.

The third group contains *Alectorolophus* (*Rhinanthus*) and part of the genus *Lathraea,* which SCHMID includes in the Scrophulariaceae. Here, as in the second group, the lower cell becomes a haustorium without septation, and two cells arising at the micropylar end of the upper cell give rise to a micropylar haustorium. Each of these cells has two nuclei.

Veronica, Euphrasia, Pedicularis, Melampyrum, and *Tozzia* are put in the fourth group, which differs from the third only in that

the micropylar haustorium arises from a single cell and contains four nuclei.

SCHMID has made it clear that the different species of a genus closely resemble one another in the character of the endosperm and haustoria, and he believes that these characters should receive consideration in drawing up a natural classification of the family. Judging things from this point of view, the Rhinantheae appear to be a natural group, since all the members studied show an extraordinary similarity of development. It certainly is striking that *Linaria* and *Striga*, which are widely separated in the present system of classification, should show such close agreement in their development of endosperm and haustoria. Though SCHMID has not paid much attention to the proembryo, it seems that here also is a strong resemblance. Surely there must be a close relationship between genera which show agreement in minute details of the development of organs which do not seem to be influenced by environment to nearly the same extent as those organs which are most used in drawing up a scheme of classification.

DOP (7) finds in *Buddleia* a remarkably close resemblance to *Verbascum*, *Scrophularia*, and *Digitalis* as regards the mode of formation of the endosperm and haustoria. He concludes that the evidence obtained emphasizes the relationship between the Scrophulariaceae and Buddleiaceae (if the latter be regarded as a family distinct from the Loganiaceae as WETTSTEIN [16] thinks it ought to be), and that it tends to separate the Buddleiaceae more widely from the Solanaceae.

The question whether parasitism of the plant as a whole affects the embryo sac and embryo has often been raised. The evidence afforded by the Scrophulariaceae all tends to show that these structures are not influenced by the habit of the plant. Extensive development of the haustoria is certainly characteristic of the Rhinantheae, but *Striga*, which is also a semi-parasite, is not so characterized, while *Veronica*, a non-parasite, has practically the same degree of haustorial development as the Rhinantheae. It seems, therefore, that intensity of haustorial development is not to be correlated with the parasitic habit of the plant.

BERNARD (2) was led to the same conclusion from a study of four total parasites: *Lathraea, Orobanche, Phelipaea,* and *Cytinus,* in which he found that all stages of haustorial development were represented. *Lathraea* shows the most extensive haustorial development of the four genera, while in *Cytinus* the endosperm is normal or nearly so.

In the production of fertile seeds the parasitic members of the family seem not one degree less successful than their independent relatives, and there is nothing in the history of the ovule of parasites to lead one to suppose that it has suffered owing to the mode of life adopted by the parent plant.

Summary

1. *Striga lutea* is a semi-parasitic annual belonging to the Rhinantheae-Gerardieae group of the Scrophulariaceae.

2. The ovary is of the ordinary bilocular scrophulariaceous type, and the ovules are anatropous, with one integument.

3. The megaspore mother cell arises directly from a single subepidermal cell, which gives rise to a row of four megaspores, of which the lowest develops into the embryo sac.

4. The 8-nucleate embryo sac develops in the normal way, and at the time of fertilization contains two synergids, an egg, the fused polar nuclei, which lie in the upper part of the sac, and three antipodal cells, which show signs of disintegrating.

5. Double fertilization occurs.

6. Endosperm is formed by cell division. From the chalazal end a long binucleate haustorium is formed, penetrating the integument. The micropylar haustorium is inconspicuous, simply consisting of a few ordinary endosperm cells with fairly dense contents.

7. The proembryo has a long suspensor of three or four cells. The basal cell of the suspensor forms tuberous haustoria.

8. The mature embryo is of the usual dicotyledonous type and is surrounded by one thick-walled layer of endosperm cells.

9. The testa consists of one layer of lignified cells which are admirably suited to protect the young embryo.

In conclusion I should like to take this opportunity for thanking Dr. H. H. W. PEARSON for his help during the early stages of this work, which was started at the South African College, and for his continued interest in its progress. I am indebted to Professor SEWARD for kind permission to work at the Cambridge Botany School, and to Mr. R. P. GREGORY for many helpful criticisms.

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PLATE VIII

MICHELL on STRIGA

MICHELL on STRIGA
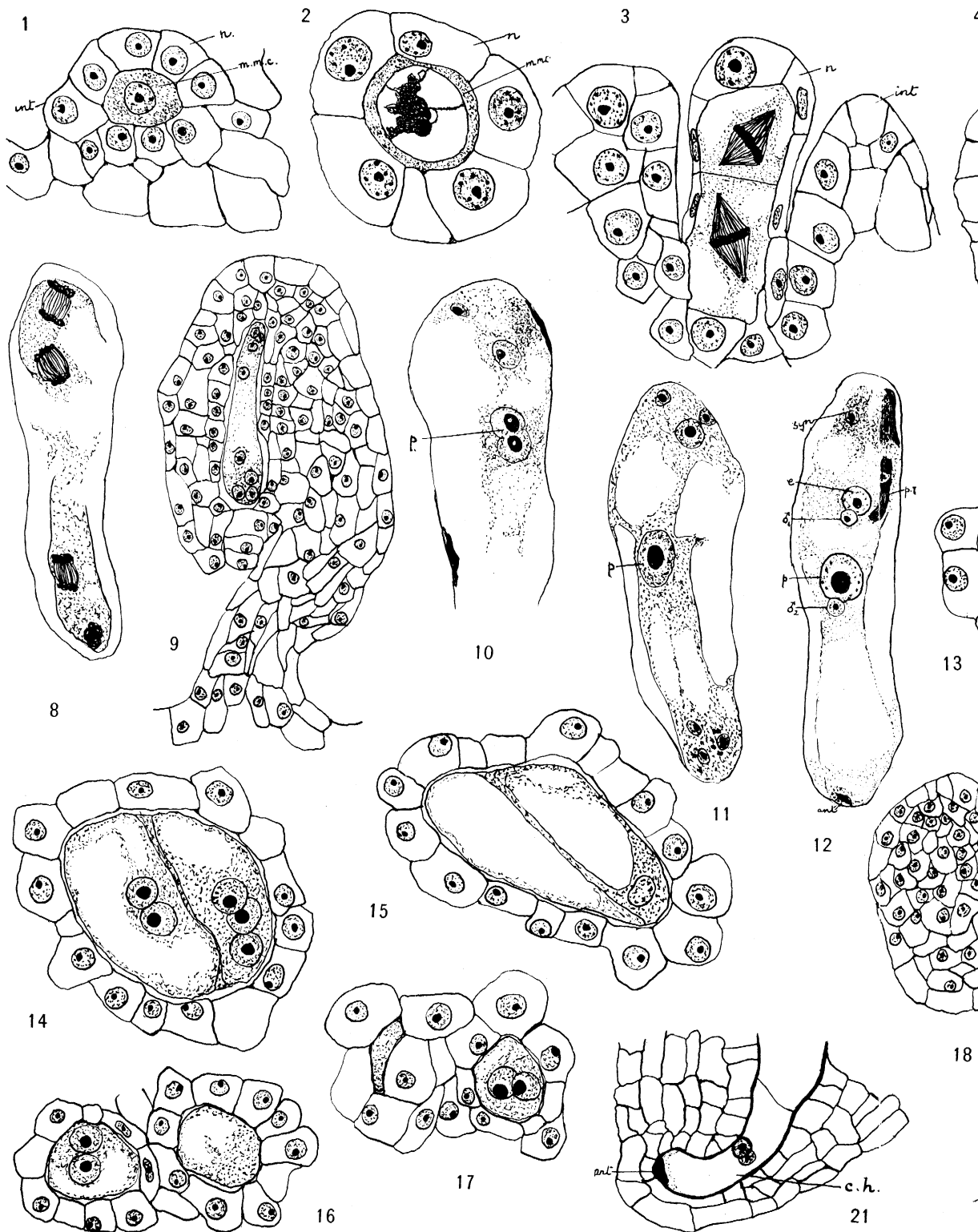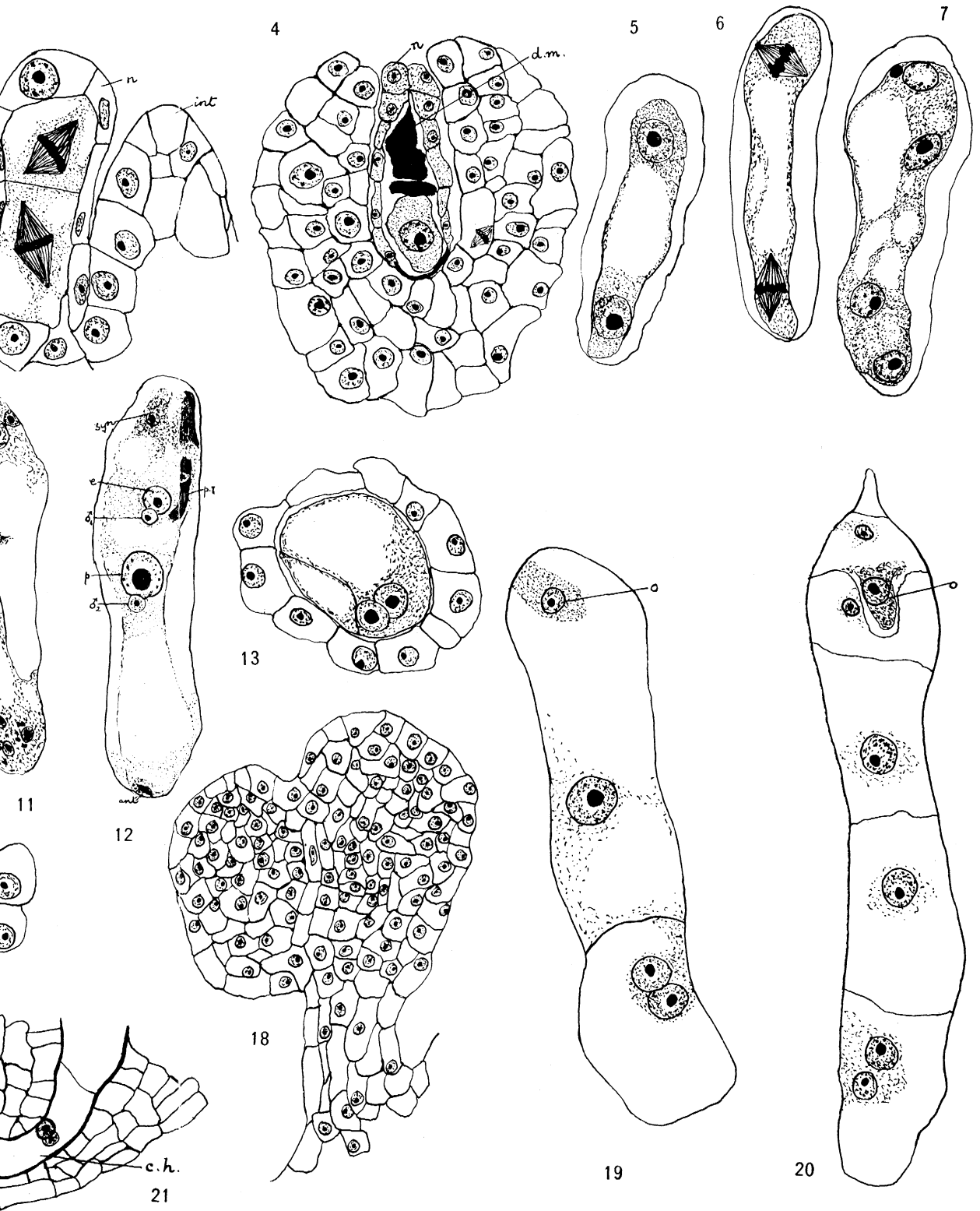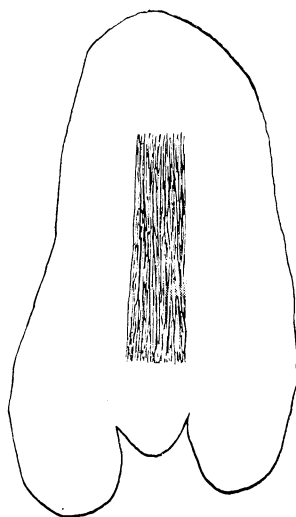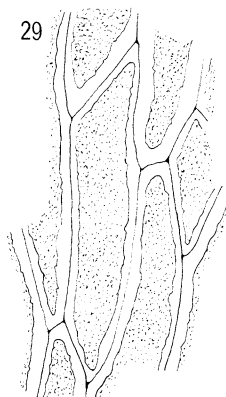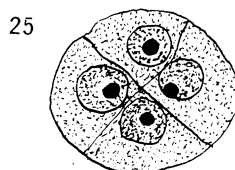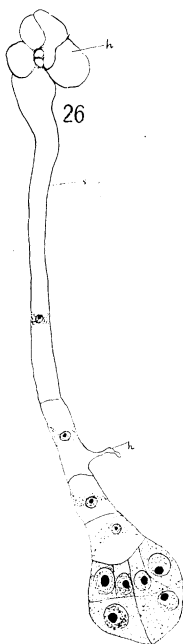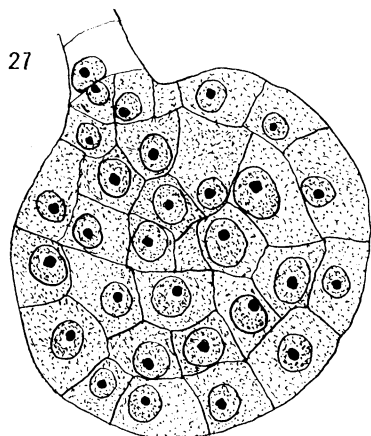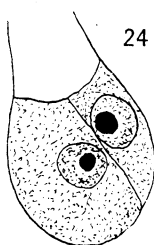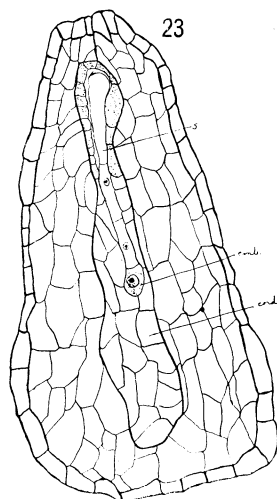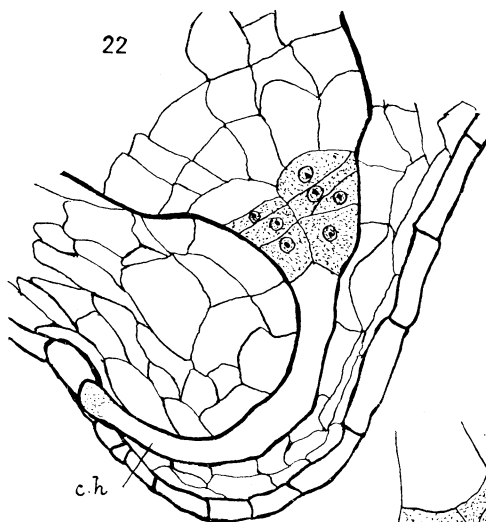
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EXPLANATION OF PLATES VIII AND IX

Abbreviations: *mmc,* megaspore mother cells; *int,* integument; *n,* nucellus; *dm,* degenerating megaspores; *p,* polars; *syn,* synergids; *ant,* antipodals; *e,* egg; *pt,* pollen tube; *o,* oospore; *ch,* chalazal haustorium; *emb,* embryo; *end,* endosperm; *s,* suspensor; *h,* haustorium.

PLATE VIII

Fig. 1.—Young ovule showing the megaspore mother cell and the origin of the integument; ×1210.

Fig. 2.—Megaspore mother cell with the nucleus in synapsis; ×1470.

Fig. 3.—Homotypic division of the megaspore mother cell nucleus; ×1470.

Fig. 4.—Ovule showing the embryo sac and three degenerate megaspores; ×1150.

Fig. 5.—Binucleate embryo sac; ×1210.

Fig. 6.—Division of the two nuclei; ×1210.

Fig. 7.—Tetranucleate sac; ×1210.

Fig. 8.—Division of the four nuclei; ×1210.

Fig. 9.—Ovule showing an 8-nucleate sac; ×1210.

Fig. 10.—Polar nuclei fusing; ×1210.

Fig. 11.—Mature embryo sac, just before fertilization; ×1210.

Fig. 12.—Fertilization; ×1210.

Figs. 13–17.—Abnormal ovule with two embryo sacs; ×1210.

Fig. 18.—Section through this ovule not showing the embryo; ×520.

Fig. 19.—After the first divisions of the primary endosperm nucleus; ×1210.

Fig. 20.—Later stage in endosperm formation; ×1210.

Fig. 21.—Part of an ovule showing the young chalazal haustorium; ×520.

PLATE IX

Fig. 22.—Older chalazal haustorium; ×520.

Fig. 23.—Proembryo imbedded in endosperm; the chalazal haustorium cannot be seen in this section; ×260.

Figs. 24–27.—Stages in the development of the embryo; figs. 24, 25, 27, ×1210; fig. 26, ×575.

Fig. 28.—Embryo from the mature seed; ×71.

Fig. 29.—Surface view of the testa of the ripe seed; ×605.